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Chromogenic agar media for rapid detection of Enterobacteriaceae in food samples

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Recently, chromogenic media were used for rapid detection of pathogenic agents isolated from different materials (food, water and stool). Results of the present study indicated that specific and rapid identification of Enterobactiriaceae isolates from 46 food samples (egg, dairy products, meat, salads and cooked rice) as compared to conventional methods of biochemical test and plating selective media consume times, are expensive , autoclaving, and less selective. Both media ECC Agar (HIMEDIA) and Salmonella differential agar (Raj Hans Medium, HIMEDIA) are powerful for screening isolates from food samples: *Esherichia coli, Salmonella* spp., *Klebsiella pneumoniea.*

Key words: Chromogenic media, Enterobacteriaceae, detection, food.

INTRODUCTION

Enterobacteriaceae, *Esherichia coli* coliform are the most infectious bacteria of foodstuff products. The detection and quantification of this emerging pathogen is therefore an important task for microbiological food and clinical diagnostic laboratories. Traditional methods for bacterial detection like biochemical test have been used for long. These methods consume time and materials. Previous study on Coliforms and specially *E. coli* made it possible to identify them as microbial contaminants marker in food and water. Their presence in drinking water and food indicates that these materials are contaminated with other enteric pathogens. So their isolation and enumeration have great importance in the determination of food hygiene (Muller et al., 2001). Standard ISO 6579 2003 (Microbiology of food and animal feeding stuffs – Horizontal method for detection of *Salmonella* spp.) includes four stages of the detection process and depending on the need to obtain confirmations, it lasts for 5 to 7 days: 1. Pre-enrichment in non-selective liquid medium; 2. Selective enrichment in liquid media; 3. Plating on selective media; 4. Serological and biochemical identification of suspected colonies

For the detection of *Salmonella* spp. as a frequent cause of gastroenteritis, there is need to isolate the pathogen from stool samples. Media containing lactose, plus a pH indicator, have been traditionally used for differentiation of Salmonella (a non-fermenter) from Commensals such as *Escherichia coli*. However, it is frequently necessary to screen many other commensals that also fail to ferment lactose (e.g. *Proteus* spp.) to

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exclude the presence of *Salmonella*. The screening of commensal bacteria to exclude pathogens is time consuming and can be costly in terms of serological or biochemical reagents (Perry and Freydie`re, 2007).

Analysis of naturally contaminated food samples showed that enumeration and isolation of colonies was much easier on the chromogenic plating media, in particular on *Bacillus cereus* group plating medium (BCM), than on the standard plating media (Martina et al., 2008). Chromogenic media contain synthetic chromogenic substrates that are cleaved by specific enzymatic activities of certain micro-organisms (Manafi, 1996, 2000; Reissbrodt, 2005).

Over the last 30 years, a range of chromogenic media has been developed that are designed to target pathogens with high specificity. Such media exploit enzyme substrates that release coloured dyes upon hydrolysis, thus resulting in pathogens forming coloured colonies that can easily be differentiated from commensal flora. Ideally, commensal bacteria should either be inhibited completely by selective agents or form colourless colonies thus allowing pathogens to 'stand out' against background flora. This allows clear differentiation of microbes producing the target enzyme from those that do not. This is especially important when attempting to detect specific pathogens within polymicrobial cultures. The substrate and products of hydrolysis should be no inhibitory to microbial growth (Ledeboer et al., 2007). The goal of this study was to evaluate the efficiency of two chromogenic media in detecting and isolating Enterobacteriaceae from food samples.

MATERIALS AND METHODS

Media preparation

Brain heart infusion broth (BHI; DifcoLabrotaries, USA), selective media were used for Gram negative bacteria, Mac Conkey Agar (FlukaBioChemika), EMB Agar (HiMedia Laboratories) and Tetrathionate Broth (OXOID) were used for coliform and enteric pathogens, whilr Bismuth Sulphite Agar (HiMedia), Hekton Enteric Agar (OXOID), XLD Agar (Becton Dickinson), and SS Agar (Biolife) were used for isolation of *Salmonella* spp.

Two chomogenic media were prepared for detection of *E. coli* and other coliforms bacteria: Hicrome ECC Agar (HIMEDIA) and Salmonella Differential Agar (Raj Hans Medium, HIMEDIA). Each medium was prepared by boiling without autoclaving.

Food sampling

Forty six samples of different foods (eggs, salad, dairy products, meet and cooked rice) were purchased from local markets in Baghdad capital in Iraq.

Bacterial isolation on different media

25 g of each sample was added to 225 ml normal saline solution; after three dilutions of 0.1ml of each food suspension were cultured separately in the different media described above .The plates were incubated at 37°C for 24 h for full color development of chromogenic media.

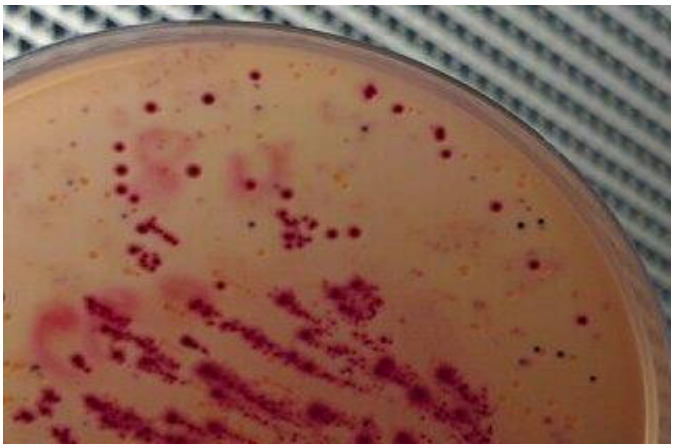

Figure 1. Stock solution of food sample on ECC Agar media.

Biochemical analysis

Exponentially growing cells of the various isolates were used for biochemical analysis in API 50 CHL kit (Biomerieux) following the manufacturer's instruction (Ramli et al., 2014).

RESULTS AND DISCUSSION

Forty six samples of different foods were examined for their bacterial micro-flora. All 46 samples contained mixtures of Enterobacteriasae, *E. coli* (n=28), *Klebsiella pneumonia* (n=7), *Salmonella* spp. (n=30). Every isolate appears with specific color on ECC & Salmonella differential agar. Stock solution of food samples contained many types of bacteria differentiated with the colors shown in Figure 1. *E. coli* isolates appear as blue and violet on ECC (Figure 2).

Salmonella spp. on two chromogenic media: *Salmonella* enteritidis is a colorless colony on ECC(A), *Salmonella* typhi & *Salmonella* typhimurium are pink colony on Salmonella Differential Agar (B) (Figure 3).

K. pneumonia is a blue colony on *Salmonella* Differential agar and green to torques on ECC (Figure 4). Preliminary identification of the isolate was based on the Gram stain. Enterobacteriaceae were detected on Mac Conkey Agar morphologically and culturally. Plated enrichment cultures from food samples grown overnight on Brain heart infusion broth, Mac Conkey Agar, EMB Agar, Bismuth Sulphite Agar, Hekton Enteric Agar, Tetrathionate Broth, XLD Agar, SS Agar media resulted in massive growth of Enterobacteriaceae, which made it difficult to isolate suspected bacteria. When comparing the chromogenic media for Enterobacteriaceae detection, ECC and *Salmonella* differential agar were found most suitable for Enterobactereiaceae members and tested

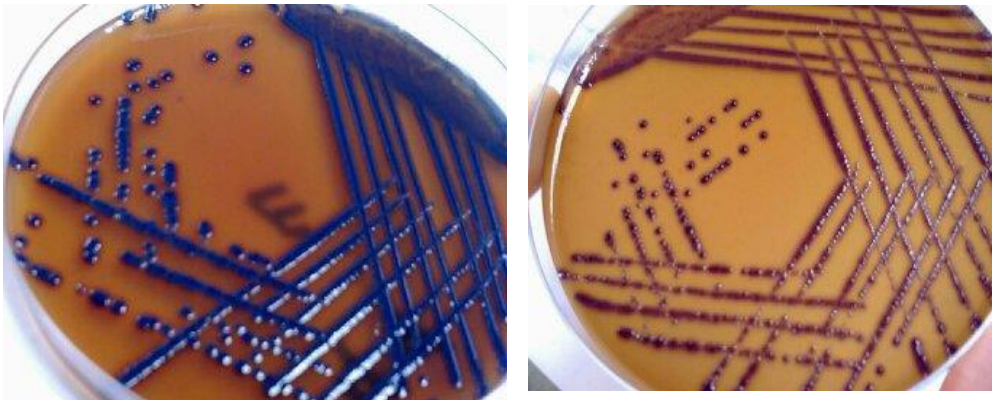

Figure 2. *E. coli* on ECC Agar media.

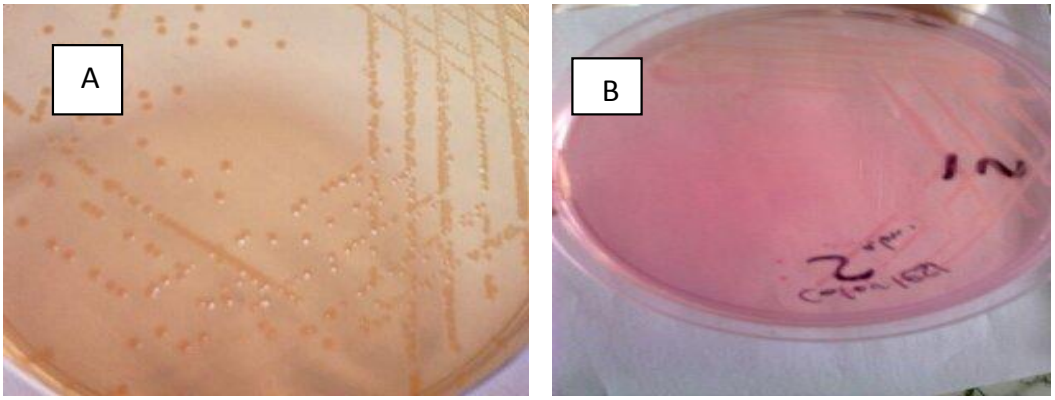

Figure 3. *Salmonella* spp. on *Salmonella* differential agar.

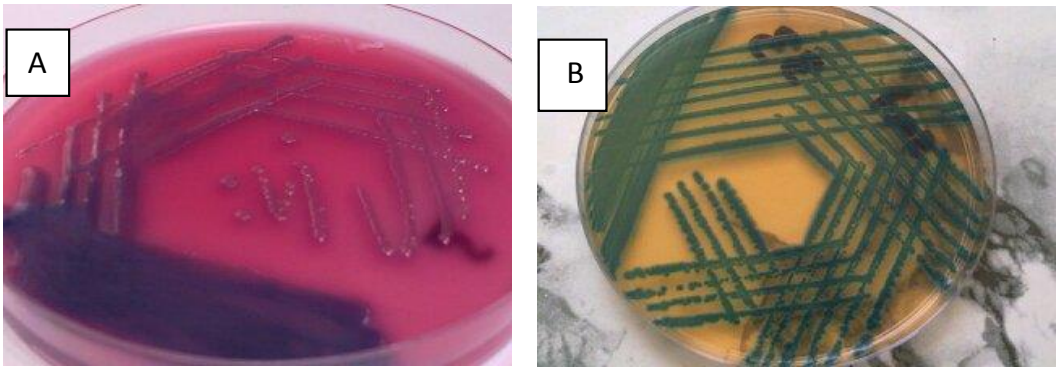

Figure 4. *K. pneumonia* (A) on Salmonella differential agar; (B) on ECC.

indicator media for the detection of Enterobacteriaceae from food samples. Replacement of Mac Conkey Agar and EMB Agarmedia by chromogenic media (ECC, Salmonella differential agar) resulted in an approximately 50% reduction of the natural bacterial background flora without decreasing numbers of detected bacterial isolates.

Interestingly, the bacterial background and flora from food sample were strongly inhibited. Salmonella colonies were isolated using chromogenic media and were

confirmed for their serotypes which is polyvalent *Salmonella* antiserum followed by specific O and H. Definitive identification of the isolates was undertaken using API 50 kits to characterize the carbohydrates – fermenting ability of the Enterobacteriaceae members. It indicated that sugars present in kit had potential to serve as discriminative markers for the selective isolation of the Enterobacteriaceae bacteria (Ramli et al., 2014).

In recent years, biotechnology advance has led to change in food test technique, and today, we benefit from methods that are more specific, faster and often more sensitive compared to conventional method (Stampi et al., 2004; Ten et al., 2004). Media containing lactose, plus a pH indicator, have been traditionally used for differentiation of *Salmonella* (a non-fermenter) from commensals such as *E. coli* (Tavakoli et al., 2008; Huang et al., 2010). The principle of chromogenic media is based on fermentation of carbohydrate that produces a localized pH drop initiating a color change in the indicator present in media. Overall we showed that two types of chromogenic media are good for rapid detection of food born bacteria; they are highly selective, need no autoclaving when compared with the conventional media that consume time, are expensive and poorly selective. These results are associated with other reports (Obeng-Nkruumah et al., 2013). Isolation and identification of food born bacteria like Enterobacteriaceae on chromogenic media were most suitable for the selection of all isolates that contribute to the color of the colonies due to the metabolic activity of these bacteria that reacted with the substrate of this media. In many studies chromogenic media demonstrate a proven advantage over conventional culture media due to a superior detection rate for target pathogens or a superior differentiation of mixed cultures. Media containing chromogenic substrates are invariably more expensive than conventional media but this can be offset by a reduced need for complementary reagents and less labor time associated with the processing of culture plates and suspect pathogens. Due to these factors, the use of chromogenic media in diagnostic laboratories is increasingly widespread. It is likely in the next few years that a wider range of pathogens will be targeted to continue the rapid expansion of the range of chromogenic media available for isolation (Paniagua et al., 2010; Ramli et al., 2014). Hicrome ECC Agar and *Salmonella* differential agar were useful media for rapid detection of food born bacteria that cause many diseases.

Conclusion

From this study, ECC, Salmonella differential agar showed high specificity and sensitivity as in other reports. However, the drawback in this study was limited to two chromogenic media used for screening Enterobacteriaceae. Nevertheless, they can be alternative for cheap routine Enterobacteriaceae to prompt the initiation of infection control measures.

Conflict of Interests

The authors have not declared any conflict of interests.

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